Optimal Intracellular Calcium Signaling

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(Received 2 October 2001; published 24 January 2002)

In many cell types, calcium is released from internal stores through calcium release channels upon external stimulation (e.g., pressure or receptor binding). These channels are clustered with a typical cluster size of about 20 channels, generating stochastic calcium puffs. We find that the clustering of the release channels in small clusters increases the sensitivity of the calcium response, allowing for coherent calcium responses at signals to which homogeneously distributed channels would not respond.

DOI: 10.1103/PhysRevLett.88.068102 PACS numbers: 87.16.Ac, 02.50.Fz, 05.40.–a, 87.15.Aa

Many important cellular functions are regulated by intracellular and intercellular Ca^{2+} signals. They are involved in the insulin production of pancreatic β cells [1], in the enzyme secretion in liver cells (for a review, see, e.g., [2]), and for the early response to injury of brain tissue [3] and corneal epithelia [4]. Recent new insights into the biophysical mechanism of intracellular Ca^{2+} release have revealed that the actual release sites are discrete and as small as about 100 nm indicating that mesoscopic methods are necessary for realistic models of Ca^{2+} release. Consequences of the discreteness of the release clusters for Ca^{2+} wave formation have been explored in [5] and [6]. In this Letter, we show that the clustering of the release channels can resonantly enhance the sensitivity of the calcium signaling pathway by exploiting internal fluctuations.

Most of the Ca^{2+} that constitutes the signal is released from intracellular stores such as the endoplasmic reticulum (ER) into the intracellular space through the Inositol 1,4,5-Trisphosphate (IP_3) receptor. The IP_3 receptor (IP_3R) is modeled [7] by three identical subunits that each have three binding sites: one for the messenger molecule IP_3 (m gate), one activating site (n gate) for Ca^{2+}, and one inactivating site (h gate) for Ca^{2+}. In order for a subunit to be conducting Ca^{2+}, only the IP_3 and the activating Ca^{2+} binding site need to be occupied. The entire IP_3R is conducting if three subunits are conducting. The Ca^{2+} binding site invokes an autocatalytic mechanism of Ca^{2+} release (Ca^{2+} induced Ca^{2+} release) giving rise to a rapidly increasing intracellular Ca^{2+} concentration if the concentration of IP_3 exceeds a certain threshold. When the inactivation Ca^{2+} binding sites become occupied and the IP_3Rs close, the Ca^{2+} pumps remove Ca^{2+} from the intracellular space, which is necessary since elevated concentrations of Ca^{2+} are toxic for the cell. Once the Ca^{2+} concentration is low and IP_3 is present in sufficient concentration, calcium induced calcium release will rapidly increase intracellular calcium levels giving rise to oscillatory calcium signals. The oscillatory nature of the Ca^{2+} signals suggests that the primary information content of the Ca^{2+} signals is their frequency [8]. In previous work it has been reported that globally IP_3-mediated Ca^{2+} signals can be devolved into localized Ca^{2+} release events due to *clustered distribution* of IP_3Rs [9] with only a few tens IP_3Rs per cluster and a size of about 100 nm, indicating that thermal open-close transitions of single IP_3Rs are essential. Observations of signals of differing magnitudes first suggested a hierarchy of calcium signaling events, with smaller blips representing fundamental events involving opening of single IP_3R and the larger sparks or puffs being elementary events resulting from the opening of small groups of IP_3Rs [9,10]. Improved spatial and temporal resolution recordings, however, have revealed that there is not a clear distinction between fundamental and elementary events [9,11]. It is suggested that the localized calcium release varies in a continuous fashion due to *stochastic variation* in both numbers of channels recruited and durations of channel openings.

In this Letter we focus on the Ca^{2+} release from a single cluster of IP_3Rs which can be considered the fundamental biophysical process in generating the Ca^{2+} signal. We are particularly interested in the periodicity with which the cluster of IP_3Rs releases Ca^{2+} since this periodicity constitutes the actual Ca^{2+} signal. The key result of this Letter is that there exists an optimal number of IP_3Rs constituting a cluster at which the periodicity of the stochastic Ca^{2+} signal is maximized. Our study is based on the Li-Rinzel model [12], a two-variable simplification of the De Young–Keizer model [7] where the fast variables m, n have been replaced by their quasiequilibrium values m_∞ and n_∞. According to this model, the calcium flux from the ER to the intracellular space is driven by the Ca^{2+} gradient; i.e.,

$$\frac{d[Ca^{2+}]}{dt} = -I_{Ch} - I_P - I_L\,, \tag{1}$$

$$\frac{dh}{dt} = \alpha_h(1 - h) - \beta_h h\,, \tag{2}$$

with

$$I_{Ch} = c_1 v_1 m_\infty^3 n_\infty^3 h^3([Ca^{2+}] - [Ca^{2+}]_{ER})\,, \tag{3}$$

$$I_P = \frac{v_3[Ca^{2+}]^2}{k_3^2 + [Ca^{2+}]^2}\,, \tag{4}$$

$$I_L = c_1 v_2([Ca^{2+}] - [Ca^{2+}]_{ER})\,. \tag{5}$$

Here, $[Ca^{2+}]$ denotes the intracellular Ca^{2+} concentration, $[Ca^{2+}]_{ER}$ the Ca^{2+} concentration in the ER, and

h a slow inactivation variable. I_{Ch} denotes Ca^{2+} efflux from intracellular stores through IP_3R channels, I_P the ATP (Adenosine Triphosphate) dependent Ca^{2+} flux from the intracellular space back to the stores, and I_L represents the leak flux. The slow Ca^{2+} inactivation process depends on both the concentration of IP_3 and Ca^{2+} via the rate constants

$$\alpha_h = a_2 d_2([IP_3] + d_1)/([IP_3] + d_3), \quad \beta_h = a_2[Ca^{2+}]. \tag{6}$$

The other parameters are $m_\infty = [IP_3]/([IP_3] + d_1)$, $n_\infty = [Ca^{2+}]/([Ca^{2+}] + d_5)$, $c_1 = 0.185$, $v_1 = 6\ s^{-1}$, $v_2 = 0.11\ s^{-1}$, $v_3 = 0.9\ \mu M\, s^{-1}$, $k_3 = 0.1\ \mu M$, $d_1 = 0.13\ \mu M$, $d_2 = 1.049\ \mu M$, $d_3 = 0.9434\ \mu M$, $d_5 = 0.082\,34\ \mu M$, and $a_2 = 0.2\ \mu M^{-1}\, s^{-1}$. The total amount of Ca^{2+} is conserved via the Ca^{2+} concentration in ER with $[Ca^{2+}]_{ER} = (c_0 - [Ca^{2+}])/c_1$ with $c_0 = 2.0\ \mu M$. The concentration of IP_3 denoted by $[IP_3]$ is a control parameter.

The form of Eq. (2) suggests that the inactivation process for each IP_3R can be modeled as a stochastic process where $h = 1$ describes the open IP_3R and $h = 0$ describes the closed IP_3R (i.e., no calcium current through the IP_3R)—constituting the *stochastic Li-Rinzel model*. The power three of h in Eq. (1) indicates the three subunits of the IP_3R and thus three inactivation h gates. For each inactivation gate h we assume a two-state Markov process with the unbinding (opening) rate of α_h and the binding (closing) rate β_h. The IP_3R is h-open if all three h sites are unbound. The Ca^{2+} flux through the IP_3R in the kinetic model is then given by the modified form of Eq. (3):

$$I_{\mathrm{Ch}} = c_1 v_1 m_\infty^3 n_\infty^3 \frac{N^{h\text{-open}}}{N} ([Ca^{2+}] - [Ca^{2+}]_{ER}), \tag{7}$$

where N and $N^{h\text{-open}}$ indicate the total number of IP_3Rs and the number of h-open receptors in the cluster, respectively. Equations (1)–(6) represent the deterministic limit of the stochastic scheme with Eqs. (1),(4)–(7) for a large number N of channels. The release of Ca^{2+} in the stochastic Li-Rinzel model is a collective event of a number of globally coupled channels (via the common Ca^{2+} concentrations) with stochastic opening and closing dynamics.

Several methods to simulate such a kinetic scheme have been put forward (see, e.g., Ref. [13]). Here we simulate explicitly each gate by the two-state Markov process with opening and closing rates α_h and β_h, respectively [14]. The differential equation (1) together with the Markov scheme for the h gates is iterated using an explicit first order scheme with a time step of 0.01 s, which is small against the time constant of the h gates $\tau_h = 1/(\alpha_h + \beta_h) > 3$ s for $[Ca^{2+}] < 1.0\ \mu M$ and $[IP_3] < 1.0\ \mu M$ and the much longer time scales in Eq. (1).

In the deterministic limit (i.e., $N \to \infty$), the two-variable Li-Rinzel model has one stable fixed point for $[IP_3] < 0.354\ \mu M$ and $[IP_3] > 0.642\ \mu M$. At $[IP_3] = 0.354\ \mu M$ and $[IP_3] = 0.642\ \mu M$ Hopf bifurcations occur so that $[Ca^{2+}]$ is oscillating for $0.354\ \mu M < [IP_3] < 0.642\ \mu M$ (Fig. 1a). Under normal conditions $[IP_3]$ is below the critical value $0.354\ \mu M$ and the deterministic model with a fixed point does not permit calcium signaling. In Fig. 1b, traces of a Ca^{2+} signal released from a cluster with 20 IP_3Rs are shown for three values of $[IP_3]$ in the three deterministically distinguished regimes I, II, III (see Fig. 1a). The Ca^{2+} signals consists of stochastic sequences of Ca^{2+} release events (calcium puffs) in all three regimes (I, II, III) with a continuum of amplitudes and durations. The regimes I, II, and III are not well distinguishable for these small clusters. Most importantly for the purpose of this Letter, the Ca^{2+} puffs for $[IP_3] < 0.354\ \mu M$ constitute a Ca^{2+} signal with a frequency content. To determine the degree of periodicity of the Ca^{2+} released from a cluster, we compute the normalized power spectrum

$$S_s(\omega) = \frac{1}{T} \frac{\left| \int_0^T \{[Ca^{2+}](\tau) - \langle [Ca^{2+}] \rangle\} \exp(-2\pi i \omega \tau)\, d\tau \right|}{\sqrt{\langle ([Ca^{2+}] - \langle [Ca^{2+}] \rangle)^2 \rangle}}, \tag{8}$$

where the length of the observation interval T is 5000 s for all data presented in this Letter. To reduce statistical fluctuations due to the finite time interval of recording, we divide the frequency axes into bins and average the power spectrum $S(\omega)$ in each bin over 300 runs. In Fig. 2, we show the normalized power spectra $S(\omega)$ at various sizes N of the release cluster. For very small clusters (e.g., $N = 2$ in Fig. 2a) and very large clusters (e.g., $N = 10\,000$ in Fig. 2c), the power spectrum does not exhibit a peak and thus the release of Ca^{2+} is dominated by stochastic events. In between, however, a peak in the power spectrum (Fig. 2b) indicates periodicity in calcium release. The *strength* of the peak is characterized by the elevation of the peak ΔS which is shown in Figs. 3a and 3b as a function of the size of the cluster N for $[IP_3] = 0.25\ \mu M$ and $0.3\ \mu M$, respectively. For $[IP_3] = 0.25\ \mu M$, the elevation of the power spectrum goes through a maximum at $N \approx 20$, while at $[IP_3] = 0.3\ \mu M$ the maximum is at about $N = 150$. Typical recorded values of $[IP_3]$ range between $0.15-0.25\ \mu M$. In this context it is interesting to note that the coherence for $[IP_3] = 0.25\ \mu M$ peaks at $N = 20$ which is considered a realistic cluster size (see also [15]).

To summarize, the overall coherence of the Ca^{2+} signal exhibits a maximum at a cluster size that depends on the concentration of IP_3. For IP_3 concentrations closer to the Hopf bifurcation the maximum coherence is achieved for larger clusters of IP_3Rs and vice versa (compare Figs. 3a and 3b). We now connect these observed phenomena with the *coherence resonance* phenomenon [16]. Here, models of excitable dynamics such as the Fitzhough-Nagumo

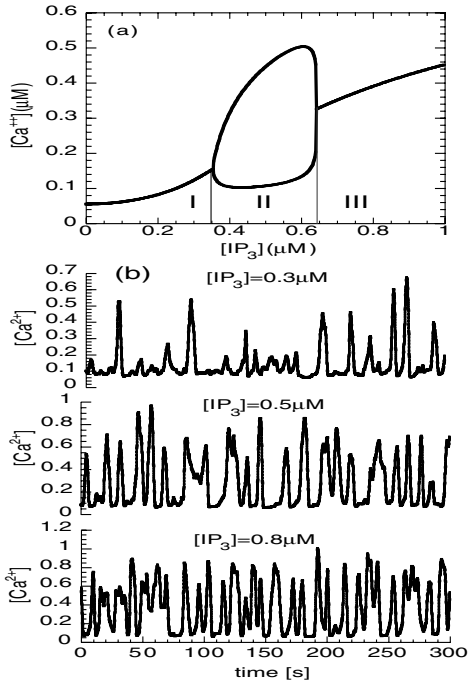

FIG. 1. The bifurcation diagram of the deterministic Li-Rinzel model (a) and calcium signals generated by a cluster of 20 IP_3Rs for different $[IP_3]$ (b).

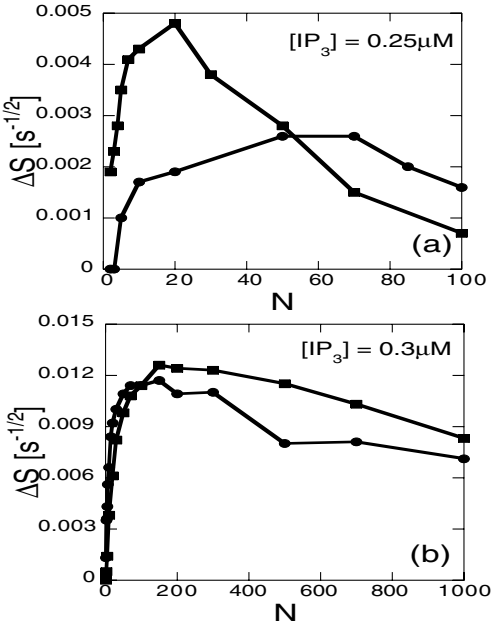

FIG. 3. The elevation of the power spectrum ΔS as a function of N at $[IP_3] = 0.25\ \mu M$ (a) and $[IP_3] = 0.3\ \mu M$ (b). Results obtained with the fully stochastic model (solid squares) are compared with results obtained from the approximative Langevin model (solid circles).

model are driven externally with noise. If the system is tuned close to the Hopf bifurcation, the resulting train of action potentials exhibits a strong periodicity if the noise strength is within a finite range of values. For Ca^{2+} release of clustered IP_3Rs, the noise is internal and determined by the *size of the release cluster*. The internal noise strength characterized by the variance of the Ca^{2+} signal is shown as a function of the size of the release cluster for $[IP_3] = 0.3\ \mu M$ in Fig. 4a. In Fig. 4b, the coherence of the Ca^{2+} release is shown as a function of the internal noise strength. The figure shows a peak, consistent with coherence resonance but with internal noise. Thus coherence

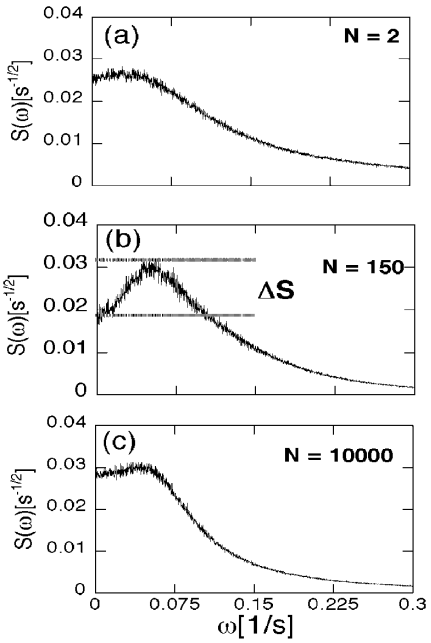

FIG. 2. Power spectra $S(\omega)$ of the Ca^{2+} signal released by clusters of (a) $N = 2$, (b) $N = 150$, and (c) $N = 10\,000$ IP_3Rs at $[IP_3] = 0.30\ \mu M$.

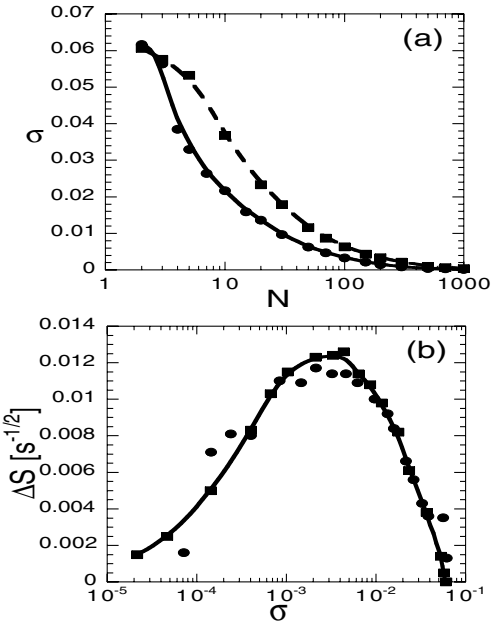

FIG. 4. The variance σ of $[Ca^{2+}]$ is plotted in (a) as a function of the cluster size. In (b) the coherence of the calcium signal ΔS is shown as a function of σ; fully stochastic model (solid squares) and Langevin model (solid circles).

resonance is an intrinsic feature of the basic biophysical calcium release mechanism of a living cell.

The stochastic method used so far is conceptually simple and very accurate but inefficient. For whole-cell or tissue models, with millions of release clusters, computational efficiency is paramount. In the following we discuss under what conditions the stochastic method can be approximated by one single Langevin equation for the fraction of open inactivation gates of one entire cluster. Since the time scales in the dynamic equation for $[Ca^{2+}]$ are the slowest, we consider the gate dynamics with constant $[Ca^{2+}]$ during one time step of iteration (0.01 sec). For each gate ($i = 1, 2, 3$) we can write down a master equation for the number n_i of IP_3Rs with open gate i [17]:

$$\dot{P}(n_i, t) = (N - n_i + 1)\alpha_h P(n_i - 1, t) + (n_i + 1)\beta_h P(n_i + 1, t) - [n_i\beta_h + (N - n_i)\alpha_h]P(n_i, t). \quad (9)$$

For a large number N, this master equation can be approximated by a Fokker-Planck equation [18] which in turn is equivalent to the Langevin equations for the fraction of activated sites $h_i = n_i/N$ [17,19]:

$$\frac{dh_i}{dt} = \alpha_h(1 - h_i) - \beta_h h_i + G_{h_i}(t), \quad (10)$$

where $G_{h_i}(t)$ are zero mean, uncorrelated, Gaussian white-noise sources with

$$\langle G_{h_i}(t)G_{h_j}(t')\rangle = \frac{\alpha_h(1 - h_i) + \beta_h h_i}{N}\,\delta(t - t')\delta_{ij}. \quad (11)$$

Since h_i have to be bound between 0 and 1, we disregard an iteration step that leads to a h_i value outside this interval. The stochastic equation for $[Ca^{2+}]$ flux through the IP_3R is given by

$$I_{Ch} = c_1 v_1 m_\infty^3 n_\infty^3 h_1 h_2 h_3([Ca^{2+}] - [Ca^{2+}]_{ER}), \quad (12)$$

which replaces the $[Ca^{2+}]$ flux through the IP_3R in Eq. (1). Instead of solving the three independent Langevin equations for h_i one can solve only *one* Langevin equation of the type in Eq. (10) (say with variable h) and then substitute $h_1h_2h_3$ in Eq. (12) by h^3. The error for the mean value $\langle[Ca^{2+}]\rangle$ due to this approximation at $N = 15$ is less than 5%, but the gain in computational speed is a factor of 3. In Figs. 3 and 4 we compare results obtained from the single-Langevin equation approach with those obtained from fully kinetic simulations. The Langevin approach produces accurate results at large N (for which it is designed) but yields only qualitative agreement for realistic cluster sizes of $N \approx 20$.

In conclusion, we have studied a simple model for calcium release from intracellular pools into the cytosol through clustered IP_3R channels. The small size of the release clusters causes the calcium release to be of stochastic nature. We have studied the coherence of the stochastic calcium signal as a function of the cluster size. At low levels of the signaling molecule IP_3 (normal physiologic condition), the coherence assumes a maximum at a certain size of the cluster, indicating optimal signaling. This calcium signal could be used for signaling and regulating other cell functions at a level of $[IP_3]$ at which the deterministic models do not permit signaling. Thus it is *the clustering of the calcium release channels* that facilitates calcium signaling at low levels of $[IP_3]$ usually present in weakly stimulated systems (e.g., by a few molecules of agonist binding, weak mechanical pressure). We have shown that this biophysical phenomenon is a form of coherence resonance [16], where the noise strength is determined by the size of the release cluster.

This material is based upon work supported by the National Science Foundation under Grant No. IBN-0078055. We have greatly benefited from discussions with Martin Falcke.

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